Evaluation of the Teratogenic Potential of Fresh-Brewed Coffee, Caffine & Aspirin in Rats 7/7/76

#6

CAFFEINE

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July 7, 1976

Dr. George W. Irving, Jr.
Life Sciences Research Office
Federation of American Societies for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014

Dear Dr. Irving:

Appended is a copy of our manuscript, Evaluation of the Teratogenic Potential of Fresh-Brewed Coffee, Caffeine and Aspirin in Rats by Paul E. Palm, Elsie P. Arnold, Peter C. Rachwall, John C. Leyczek, Kenneth W. Teague and Charles J. Kensler, which we believe will be of interest to you since it represents the most recent information on this subject and is the study which I understand various members of the National Coffee Association have discussed with you. The data were presented in part at the 15th Annual Meeting of the Society of Toxicology, March 14-18, 1976 in Atlanta, Georgia, and the manuscript has been submitted for publication in *Toxicology and Applied Pharmacology*.

If you have any questions regarding this study we will be happy to answer them.

Very truly yours,

Paul E Palm

Paul E. Palm, Ph.D.
Head, Toxicology Laboratories

PEP/mlm

Encl.

cc: Dr. Corbin I. Miles, FDA ✓

EVALUATION OF THE TERATOGENIC POTENTIAL OF FRESH-BREWED COFFEE, CAFFEINE AND ASPIRIN IN RATS[1,2]

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Running Title:
Teratogenicity of Coffee, Caffeine and Aspirin

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Evaluation of the Teratogenic Potential of Fresh-Brewed Coffee, Caffeine and Aspirin in Rats. Palm, P.E., Arnold, E.P., Rachwall, P.C., Leyczek, J.C., Teague, K.W. and Kensler, C.J. (1976). Toxicol. Appl. Pharmacol. , - .

Dilutions of fresh-brewed coffee, at 12.5%, 25% and 50% resulting in caffeine intakes of ∿ 9, 19 and 38 mg/kg/day, respectively were consumed by female rats as their sole beverage for 5 weeks prior to mating, throughout gestation and in representative animals, until Day 37 after parturition. Other rats received a daily dose of 30 mg/kg of caffeine or 125 mg/kg of aspirin by intubation, or 30 mg/kg of caffeine in the water. None of these regimes interfered with the normal behavior, growth, patterns of eating and drinking or reproductive performance. In fetuses from aspirin-treated rats the incidence of bowed fibula and shortened humerus was 4.3% and 2.7%, respectively, and in the caffeine-intubated group, the incidence of absence of the supraoccipital bone was 5.8%, all compared to 0% in H_2O controls. No teratogenic effects were observed in the coffee-treated groups or the caffeine-in-water group. There was an apparent slight delay in ossification in fetuses of all treated groups, some kidney-pelvis underdevelopment in the 25% and 50% coffee, caffeine-in-water and aspirin groups, and slightly lower organ weights in the 50% coffee and aspirin groups. Offspring raised to maturity had no gross abnormalities of growth, development or reproductive capability.

Index Terms:

Coffee

Caffeine

1,3,7-Trimethylxanthine

Aspirin

Rats

In-Utero

Teratogenesis

Reproduction

Growth

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Caffeine is a component of several of our more popular beverages including coffee, tea, colas and chocolate as well as several commonly used over-the-counter drugs. Therefore it is appropriate that its safety, including possible teratogenic effects, be evaluated.

Caffeine has been shown to cross the placenta and enter fetal tissues in the human (Goldstein and Warren, 1962) and uterine secretions of mice, rats and rabbits (McLachlan et al, 1969); and with a number of its radioactive metabolites, enters the rabbit blastocyst following an oral dose of only 3.5 mg/kg to a six-day pregnent dam (Fabro and Sieber, 1969).

Early studies of the teratological potential of caffeine commonly utilized a massive dose administered to pregnant rodents as a single injection by a variety of routes (Nishimura and Nakai, 1960; Fujii et al, 1969 and Snigorska and Bartel, 1970). Repeated oral intubation of 75 mg/kg caffeine 6 days/wk (Day 5-sacrifice) produced anomalies in Swiss mice in two of five collaborating laboratories (Group d'etude, des risques teratogenes, 1969) while 50 mg/kg (Days 5 or 6-18) died not (Bertrand et al, 1965 and 1970). However, one malformation (adactyly) in 38 fetuses versus 0/1000 in the control group (Bertrand et al, 1965), and resorptions (Bertrand et al, 1970) were observed in BALB/c mice at this dosage. NMRI mice given 50 mg/kg/day of caffeine orally from Day 14 before conception to term also showed malformed fetuses, (Knoche and Konig, 1964). CD-1 mice intubated with up to 36 mg/kg of caffeine on Days 6-15 of gestation showed no dose-related teratological effects (Food and Drug Research Labs., Inc., 1973 A).

Incorporation of a 180 mg/kg/day dose in the diet of Sprague-Dawley rats throughout gestation failed to produce malformed fetuses, but did increase the incidence of resorptions, edema and low birth weight. However, malformations (esophagectopies) were noted in rats which consumed diets containing a caffeine dose of 300 mg/kg on Days 8-14, but not when given this dose on Days 1-7 or 15-21 (Fujii and Nishimura, 1972). In Wistar rats, an increase in the incidence of malformations was recorded after daily doses of 75, 100, 125 and 150 mg/kg administered from Day 8 before conception to term (Bertrand et al, 1965), and after 125 mg/kg given on Days 2-15 of gestation (Bertrand et al, 1970). Oral intubation of rats with caffeine doses up to 22.5 mg/kg on Days 6-15 of gestation, however, produced no dose-related teratological effects (Food and Drug Research Labs., Inc., 1973 A). This study included hamsters intubated with up to 30 mg/kg of caffeine on Days 6 to 10 of gestation which also produced no malformations.

In rabbits teratological effects (absent thumbs) were detected by Bertrand et al, (1970) in 6/64 fetuses after daily oral dosing of 100 mg/kg of caffeine on Days 1-25 of gestation. Following intubation of Dutch-belted rabbits with a 32.5 mg/kg/day dose of caffeine on Days 6-18, there appeared to be an increase in maternal mortality with a decrease in the fertility rate, but no evidence of teratogenesis (Food and Drug Research Labs., Inc., 1973 B). The investigators also reported that intubation with up to 7 mg/kg on this regimen produced no clearly discernible effect on the incidence of anomalies.

The above data indicate that during gestation, repeated oral doses of 50-100 mg/kg/day of caffeine (equivalent to ∿ 38-76 cups of coffee/day in man) are required to induce any teratological effects in the laboratory animals studied.

However, the conditions of these tests do not reliably duplicate the human intake of caffeine in beverages.

The objectives of the present study were to stress rats continuously by presentation of fresh-brewed coffee for ad libitum consumption prior to and throughout gestation, and thus to determine whether coffee had effects on food and liquid consumption, body weight, fertility, number of resorptions, litter size, survival rate, growth rate of offspring, sex ratio, the incidence of soft tissue and skeletal teratological abnormalities, and the reproductive performance of "untreated" F_1 generation rats from treated dams.

Prior to initiation of the teratology study, single-dose oral intubation studies were conducted to determine the LD_{50} value of caffeine in the strain of rats to be utilized, and for comparison in the mouse, hamster and rabbit. The resulting acute toxicity data also were used to select a dosage which would be appropriate for repeated administration to pregnant dams by intubation or in the drinking water for comparison of effects of caffeine as found in coffee.

METHODS AND MATERIALS

Coffee - Ground coffee meeting the green coffee mix, roast color, grind and freshness criteria that would most nearly represent the commercial coffee commonly purchased in the United States, was provided, vacuum-packed in one-pound cans, by the National Coffee Association. Each day coffee was freshly brewed in a 100-cup electric percolator[4] beginning with 648 g of ground coffee and tap water at a temperature of 70°F (filled to the 100 cup mark) and percolated for exactly 47 minutes with an essentially consistent final temperature of 165°F.

This carefully prepared coffee and brewing procedure, which are described in detail elsewhere[5], resulted in brews considered to be representative of the brewed coffee beverage most commonly consumed in the United States.

Typical analyses showed 0.90% (± 0.034%) brew solids, caffeine 0.056% (± 0.001%), pH 5.04 (± 0.064) and brew titratable acidity of 13.56 mg (± 1.23 mg). Further analyses[6] of a coffee sample pooled from two independent brews, showed (mg/liter): copper 0.022, iron 0.30, lead 0.003, calcium 32.4, zinc 0.067, selenium < 0.004, magnesium 60.0, potassium 647.0, sodium 36.8, niacin 4.56, riboflavin 0.31, and vitamin B12 < 0.020 mcg/liter, folic acid < 20.0 mcg/liter.

The coffee was allowed to cool and then diluted with cool tap water to provide concentrations of 12.5%, 25% and 50% coffee. The dilute coffee mixtures were poured into individual glass bottles equipped with ATCO[7] stainless steel ball point (no drip) tubes and weighed prior to attachment to the individual cages at about 2:00 p.m. and again when removed the following day at 10:00 a.m. for washing and refilling.

Caffeine - Anhydrous caffeine (1,3,7-Trimethylxanthine)[8] was dissolved in distilled water and maintained in solution utilizing a magnetic stirrer at a concentration of 50 mg/ml for administration by gavage. When offered in the drinking water the concentration was adjusted according to the group mean water intake of the preceding day to provide a dosage of ∿ 30 mg/kg/day.

Aspirin - Aspirin, USP powder[9] was suspended in water at a concentration of 125 mg/ml for dosages of 250 and 125 mg/kg by intubation.

Water - Tap water available to the public in the Cambridge, Mass. area was offered daily to the negative control animals, utilized as a vehicle for the caffeine and aspirin administered to the positive control animals, and for preparation of the coffee solutions. A typical analysis of this water as reported by the Massachusetts Department of Public Health (Cambridge) showed the following (mg/liter): Hardness ($CaCO_3$) 49.0, Calcium 14.0, Magnesium 3.1, Sodium 37.7, Potassium 2.0, Iron 0.09, Manganese 0.03, Silica 3.9, Sulfate 26.0, Chloride 53.2, Nitrogen (Ammonia) 0.03, Nitrate 0.4, Nitrite 0.003, Copper 0.02, Alkalinity Total ($CaCO_3$) 22.0 pH 6.9 and Spec. Cond. (Microhms/cm) 244.2

Animals - Young adult Swiss CD mice and Sprague-Dawley CD rats[10], New Zealand white rabbits[11] and Golden Hamsters[12] of both sexes assigned to the acute toxicity studies were housed individually in wire mesh cages in air-conditioned rooms. Water, the caffeine solution or dilute coffee and Purina Lab Chow were available for consumption ad libitum. Female rats used in the teratology study were housed singly, except during mating, in plastic breeding cages. Males were maintained individually in galvanized wire mesh cages.

Preliminary Studies of the Acute Toxicity of Caffeine - Randomized groups of 15 male and 15 female mice, rats and hamsters and 10 male and 10 female rabbits were given a single dose of caffeine by oral intubation following a sixteen-hour fasting period. Five or six dosage levels were utilized per species. The animals were observed for mortality and body weight changes, 15 or 29 days after dosing. The LD_{50} was calculated by the Cornfield and Mantel (1950) modification of Karber's method.

Teratology Study - Randomly selected groups of 25 healthy young virgin female rats showing a mean body weight of 151.6 (133.0-184.0) g were provided with dilutions of fresh-brewed coffee at 12.5%, 25% or 50% as the sole source of fluid for five weeks prior to breeding and throughout gestation. Males weighing 162.5 (132.0-203,0) g were introduced to the females at about 4:00 p.m. and removed the following morning by 9:00 a.m. No treatment was given to the females during mating to the untreated males, but was resumed on evidence of pregnancy as determined by plug formation and vaginal smears Day 0. Following mating additional randomly selected groups of 25 females were given 10% of the single-dose LD_{50} level of caffeine in the drinking water, 10% of the LD_{50} level of caffeine daily by oral intubation (to permit a comparison of effects from rapid vs slower consumption of caffeine), or 125 mg/kg of aspirin daily by oral intubation. Initially, another group was dosed with 250 mg/kg of aspirin daily, but the animals rapidly succumbed. Two groups of 25 females each were given tap water throughout the experimental period to serve as negative controls. However, since no statistically significant difference could be detected between the two control groups in any of the parameters monitored, the groups were combined for comparison with all of the treated groups. Body weight of individual animals was determined weekly and fluid (daily) and food (weekly) intakes were monitored in all groups both preceding and during gestation. On the nineteenth day of pregnancy Caesarean-sections were performed and the number of live and dead fetuses and resorptions and their positions were noted. All fetuses were weighed, measured for crown-rump and transumbilical distances, and examined carefully under a dissecting microscope for gross external anomalies. One-third of the fetuses of each dam were fixed in Bouin's solution and later dissected using a modified version of the Wilson (1967) and Monie et al (1965) techniques to detect visceral anomalies; the remaining two-thirds were preserved

in buffered formalin, cleared and subsequently stained with alizarin red-S to aid in the detection of cartilage and bone anomalies, as suggested by Monie et al (1965), and stored in glycerine. Litters were examined in random order. As an additional, indirect evaluation of bone calcification, the density of alizarin red-stained bones (humerus at the deltoid process) was estimated using an A.O. Spencer monocular microscope equipped with a B & L lamp, neutral and red filters (wave length comparable to that of the alizarin red stain), and a Photovolt densitometer to measure light transmittance. Background readings with and without each filter were recorded to aid in determining the effect of the red stain on bone density readings. The fetal humerus was selected as representative of the skeletal system primarily because it could be positioned. easily in the test system. Calcium concentrations (% ash) of foreleg, hindleg and skull bones from adult rats were obtained with a Perkin-Elmer Model 503 Atonic Absorption Spectrophotometer by direct comparison to Fisher-certified calcium AA Standard.

At least 5 females in each treatment group plus 10 females in the H_2O control group were permitted to deliver their litters normally and rear their young in order to observe litter size, pup size and weight, body weight gain, the rate of survival, and gross external abnormalities. During the nursing period the coffee-treated females continued to receive coffee to Day 37, but the caffeine-and aspirin-treated groups were given no further treatment. Following weaning the F_1 generation offspring were given tap water with no treatment. At approximately 100 days of age the F_1 females were bred to randomized F_1 males to evaluate their ability to reproduce. The females were allowed to deliver their young normally. The number of live and dead F_2 pups, their mean body weight, the incidence of gross abnormalities and the survival rate to Day 28 were noted.

RESULTS

Acute Toxicity of Caffeine

In all four species tested, death occurred most frequently on the day of dosing or on the following day. Occasionally, death was delayed up to 14 days. There were few clinical signs of toxicity noted; diarrhea, sometimes severe in degree, was observed prior to death in some animals. At the highest dose levels all species showed reduced body weight gain or loss of weight during Week 1 after dosing. Generally there was recovery during Week 2. As shown in Table 1, the oral LD_{50} was highest in the rat, comparable in the hamster and rabbit and lowest in the mouse. The mouse and hamster differed from the other two species in showing more deaths among the males than among the females although the difference between the sexes was not statistically significant (ζ-test for equality of proportions). In the rat, the number of females succumbing was significantly greater than the number of males. Rabbits also had more deaths among females, but the difference between sexes was not statistically significant. At autopsy, rabbits showed slight to severe congestion of the gastrointestinal tract, particularly in the stomach, duodenum and jejunum. Occasionally, pulmonary congestion also was observed. Other tissues appeared normal.

Teratogenicity Study

P-Generation

Female rats consuming dilutions of fresh-brewed coffee as the sole fluid source for about five weeks before pregnancy and during gestation showed no detectable change in behavior or other gross signs. Prior to pregnancy these females consumed a small, but significant (Student-t) excess of fluid (1.0-5.1 ml

representing an increase of 3.1-15.7%) compared to those females consuming only water. This could not be demonstrated for each coffee level when considered alone, but significance was observed when data from all coffee levels were combined for statistical analysis. All of the rats consumed more fluid during gestation $\bar{M}$ 45.3 (33.1-59.0 ml/day) than prior to pregnancy $\bar{M}$ 33.6 (25.9-43.7 ml/day), but there was no statistically significant differences between the control and treated groups, and caffeine intake, on a mg/kg basis, remained essentially constant throughout the study. Daily caffeine intake averaged about 9 mg/kg, 19 mg/kg and 38 mg/kg in the 12.5%, 25% and 50% groups, respectively. Weekly body weight gains were comparable in all groups as were food consumptions except for the positive control group receiving aspirin. This group consumed significantly less food (Student-t test) than did the H_2O controls during Test Week 2, prior to mating (120.4 g/wk compared to 171.3 g/wk) but consumed significantly more food (172.9 g/wk compared to 121.8 g/wk) the third week of gestation.

Reproductive performance of females delivered by Caesarean section as indicated in Table 2 by mean days to pregnancy, mean number of resorptions, mean number of fetuses per litter, mean number of dead fetuses/litter, male/female ratio, and mean fetal weight, was generally comparable in all groups. A significant increase in the mean days to pregnancy, in both groups later given 30 mg/kg/day caffeine and in the aspirin group, appears to be an incidental finding since these groups were not treated in any way which was different from that of the control group prior to pregnancy. Reproductive performance also appeared normal in the forty females allowed to deliver normally (Table 3). This will be discussed in more detail in the next section, F_1 generation.

Fetuses derived by Caesarean section from dams in the various treatment groups were similar in body weight to those of the controls (Table 4). Fetal size and organ weights were generally comparable; statistically significant differences were occasionally observed, but were not clearly dose-or treatment-related. When compared with control values mean weights of fixed fetal brains, lungs and liver appeared slightly but significantly lighter, as a percent of body weight, at the 50% coffee level, but not at the 25% or 12.5% levels. Similar findings were observed in the brains and lungs of fetuses from aspirin-treated dams which in addition showed a slightly heavier fetal heart. Other treatment groups also showed apparently sporadic findings.

Gross examinations for soft tissue anomalies revealed a significantly increased incidence of kidney-pelvis underdevelopment in fetuses of the 25% and 50% coffee groups, the aspirin group and the caffeine-in-water group (Table 5). Cleft palate was more common in coffee consuming groups and the aspirin group than in the H_2O controls, but in the coffee groups there was no dose-relationship with occurrence. The incidence of cryptoorchism was significantly increased only in the groups receiving caffeine. Hydrocephaly and crooked tail were more common in the H_2O controls than in the test groups. Therefore, except for the kidney-pelvis underdevelopment, the overall incidence of soft tissue anomalies appeared similar in all groups and did not suggest a definite effect from the test materials.

The incidence of three skeletal anomalies appeared to be significantly increased and related to the test materials as shown in Table 6. Bowed fibulae were observed in 8/188 fetuses (4.3%) in the aspirin group and a shortened humerus in 5/188 fetuses (2.7%) in this same group. Absence of the

supraoccipital bone was observed in 10/171 fetuses (5.8%) from rats intubated daily with 30 mg/kg of caffeine. These anomalies were not detected in any of the 256 H_2O control fetuses. The fibula was significantly shortened in 4/141 fetuses (2.8%) from the 25% coffee group, but this did not appear to be dose-related since the 50% coffee group showed no such effect.

An increase in the incidence of irregular and incomplete ossification, as indicated by the patterned deposition of alizarin red stain binding calcium, was frequently observed in various portions of the skeletal system in fetuses from all test groups, but the incidence did not appear to be dose-related. The incidence of this effect was further analyzed statistically by combining the data to show the total number of fetuses in all test groups with any ossification abnormality. In this analysis, only the 25% coffee group appeared statistically significant while the 12.5% and 50% groups showed no significant variance from the incidence observed in the H_2O-control group.

In an effort to better understand this apparent delay in calcification of specific portions of the skeletal system, and to eliminate, as best we could, any possible bias by the observer or difference in staining with alizarin red, an estimation was made of the density of the fetal humerus from data obtained with a densitometer and appropriate light filters. As noted in Table 7, resulting data showed a statistically significant reduction in bone density in all treated groups. There was some suggestion of a dose-response relationship with coffee consumption.

$\underline{F_1 \text{ Generation}}$

At birth the number of F_1 pups/litter and the mean body weight of the pups

in the coffee groups, and the caffeine-in-water and aspirin groups were similar to that observed in the H_2O control group as reported earlier in Table 3. The group receiving caffeine by intubation had somewhat more pups per litter with slightly lower body weights, but this was not considered to be related to the test material. At 38 days of age the percent of pups surviving and their weight were similar for all treatment groups, even though dams in the coffee groups continued to receive coffee to weaning.

These F_1 rats were permitted to mature and were mated with other F_1 animals at about Day 100. Food intake before pregnancy and during gestation showed no indication of any effect due to the treatment of the parents. Water intake of the F_1 female offspring of coffee-treated females was slightly but not significantly lower than the other groups before pregnancy, but was similar to the other groups during gestation. A statistically significant lower body weight was observed in the females of the 50% coffee group at breeding and at Day 1 of pregnancy. However, by the end of pregnancy there was no difference in body weight between any of the groups.

Table 8 shows the reproductive performance of 192 "untreated" F_1 generation female rats selected at random from the various treatment groups; there were 51 from H_2O control dams and 15-29 rats from each of the other treatment groups. Pregnancy rates were similar in the coffee groups and H_2O controls, but somewhat lower in the caffeine and aspirin groups. Time to pregnancy was significantly longer, 2-9 days, in all "treated" groups except the 12.5% coffee group. This did not appear to be specifically related to the test materials since a similar finding was observed in their parents prior to any treatment. No difference was noted in any treatment group in the mean number of pups/litter at birth or

the mean body weight of the pups when analyzed by analysis of variance. Similarly there was no difference in the number of pups dead at birth and all groups appeared equally capable of raising their young. There also was no significant difference in the percent surviving or in the mean body weight of the pups at Day 28. Therefore, we conclude that under these test conditions coffee as administered to the P generation female rats prior to, throughout gestation, and for 28 days thereafter during lactation appeared to have no effect on the health or the reproductive performance of the F_1 offspring.

DISCUSSION

In the acute toxicity studies reported, the finding that lethal effects of a single, large dose of caffeine were more pronounced in Sprague-Dawley female rats than in males is particularly interesting in light of the report by Peters and Boyd (1966) that sublethal signs of toxicity in Wistar rat male survivors were greater than in female survivors intubated daily for 14 days with an oral dose of 185 mg/kg. Caffeine also was found to be more toxic in older than in younger rats. The observations by Beliles (1972) that there were no remarkable differences between pregnancy and non-pregnant animals injected intravenously with caffeine in respect to LD_{50} values should be kept in mind in evaluating the results of the present study.

The rate at which caffeine is consumed, of course, has great influence on the effects observed. For example, Leuschner and Schwerdtfeger (1968) reported that oral intubation of 130 mg/kg of caffeine Days 6-16 of pregnancy produced anomalies in fetuses of Br 46-Wistar II rats, but not when the dose was administered in the food and consumed over a 24-hour period.

The present study represents an effort to evaluate the teratogenic potential of fresh-brewed coffee in rats in a manner which more closely duplicates the human intake of caffeine in beverages. To our knowledge, no previous study of possible teratogenic effects has utilized the test method of offering, ad libitum, fresh-brewed coffee as the only source of fluid at levels in and above the range (on a mg/kg basis) of human daily ingestion of coffee. The fresh-brewed coffee was diluted to levels of 12.5%, 25% and 50%, yielding caffeine intakes of approximately 9, 19 and 38 mg/kg/day, respectively. For a 70 kg person these levels would be comparable to the consumption of about 8, 16 and 32 cups of coffee daily, (assuming 84 mg of caffeine/150 ml cup) thereby representing a range from a high normal to an extreme and abnormal intake.

It is important to note that with the coffee and caffeine levels offered, the spontaneous drinking and eating habits of the rats appeared to be essentially unaltered. That is, no statistically significant difference could be demonstrated between the H_2O control animals and any treated group in respect to mean daily fluid intake or mean weekly food consumption either before pregnancy (when only the coffee groups were treated) or during gestation. Some minor alteration in these habits was suggested during the two-week period prior to pregnancy (only when all coffee treated groups were combined for statistical analysis), but not during gestation. Further, the body weights of all coffee-treated groups were comparable to the controls throughout the study.

It was possible to obtain these high levels of intake of caffeine in rats, based on body weight, because coffee was the only liquid offered to the animals and because the relative daily water consumption of the rat ($\sim$ 140 g/kg from our data which confirm that of Adolph, 1956) is three or four times that of

man (~ 35 g/kg) as reported by Adolph (1956). However, the possible toxic or teratogenic potential of these increased levels must be viewed in the presence of other considerations such as the rate of elimination of caffeine from the blood plasma in rats which may be somewhat less than twice as fast as in man. This estimate is based on values for the rat adapted from a paper by Czok (1969) suggesting a half-life of about 1 1/2 hours, and data currently being collected at Arthur D. Little, Inc. which suggest the value may be closer to 2 1/2 hours (A. Burg, personal communication), as compared to 3 1/2 hours in man (Axelrod and Reichenthal, 1953).

Reproductive performance of the rats, as indicated by fertility, mean number of resorptions, ability to carry a litter to term, litter size and weight, male/female ratio and ability to rear the pups to weaning, was not affected by ingestion of coffee or caffeine throughout gestation and nursing at the levels tested. Nor was there any detectable effect on the reproductive performance of male or female F_1 rats which had been exposed under these test conditions to caffeine or coffee in utero and until weaning, Day 37. These observations are consistent with the previously reported failure to note changes in reproductive capabilities of three generations of mice exposed continuously to caffeine in the drinking water (Thayer and Kensler, 1973). In this earlier study the caffeine levels ingested were 4-5, 12-19 and 25-39 mg/kg/day, equivalent to about 4, 12 and 25 cups of coffee daily for a 70-kg person, a range similar to that in the present study.

It should be noted, however, that our findings are at variance with those of Gilbert and Pistey (1973) who reported that repeated intraperitoneal injections of caffeine given to Holzer rats Days 0-20 of pregnancy in dosages

ranging from 20-80 mg/kg/day resulted in significant resorptions and decrease in the birth weight of the offspring. This variance may result from the different strain of rats employed or the different route of administration utilized. The fact that these investigators administered the total daily dose in four injections, one each 6 hours, would not seem likely to have produced the difference in results because our dosage regimens included one group which received ∿ 30 mg/kg of caffeine as a single daily oral intubation and another group ad libitum in the water. Nevertheless, they too did not observe developmental malformations.

Teratogenic effects described as bowed fibulae (4.3%) and shortened humeri (2.7%) were observed with aspirin and in 5.8% of the fetuses from caffeine intubated dams an absence of the supraoccipital bone was observed. In the coffee-drinking and caffeine-in-water groups, an examination of both soft and skeletal tissues revealed no dose-related teratological effects due to coffee or caffeine consumption by the dams, although there was some evidence that the rate of tissue development was delayed slightly. Similarly Fujii and Nishimura (1972) reported the ossification was retarded in fetuses from Sprague-Dawley rats fed diets containing 0.5% caffeine during Days 1-7, 8-14, and 15-21 of gestation, but was most severe in the Day 15-21 group. However, no data were presented to suggest why they concluded the ossification was merely retarded or for how long.

Data from an ongoing "life-time" study in Sprague-Dawley rats in our laboratories, however, do support our opinion that the apparent lower calcium content (and density) noted grossly in bones of fetuses from rats receiving coffee at dilutions of 12.5%, 25% or 50% prior to and during gestation is only

a retardation in bone calcium deposition, and not a teratogenic effect. Specifically, bone calcium analyses using atomic absorption methods were conducted in a coded and randomized procedure on foreleg, hindleg and skull bones from 10 ♂ and 10 ♀ rats/level; rats which were derived from coffee-consuming dams and which subsequently were administered fresh-brewed coffee as their only beverage for one year. Bones from 20 ♂ and 20 ♀ H_2O control animals on this study also were analyzed. As noted in Table 9, no statistically significant difference was detected in the calcium content (% ash) of bones from the H_2O control rats and those which from birth had consumed ad libitum for one year 25% or 50% fresh-brewed coffee as their sole source of liquid. However, bone calcium still was slightly lower in females, but not males, which had consumed 100% coffee during this period. These data suggest that the apparent calcium deficiency observed in fetuses from rats consuming 12.5%, 25% and 50% coffee is physiologically corrected within one year or less even while coffee consumption is continued.

We conclude, therefore, that, in the present study, some apparent delay in development, including calcification of fetal bones was evident, but no teratological effects were found due to coffee consumption, and in the F_1 animals permitted to mature, no gross anomalies were observed and no treatment-related difference in body weight gain, food or water consumption or reproductive performance could be detected.

TABLE 1

SINGLE-DOSE LD_{50} VALUES FOR CAFFEINE

ADMINISTERED BY THE ORAL ROUTE IN VARIOUS SPECIES

	MOUSE			HAMSTER			RAT			RABBIT		
SEX	M	F	M + F	M	F	M + F	M	F	M + F	M	F	M + F
Number/Dose Level	15	15	-	15	15	-	15	15	-	10	10	-
Mean Body Wt (g)	33	27	-	131	141	-	210	164	-	1110	1120	-
(Range)	(25-40)	(25-30)	-	(110-150)	(116-180)	-	(190-231)	(140-186)	-	(550-1770)	(680-1980)	-
LD_{50} (mg/kg)	127	137	132	230	249	239	355	247	296	246	224	235
95% Conf Limits	114-142	122-154	122-143	210-253	225-375	224-256	312-403	220-277	282-326	229-264	211-238	224-246
Slope	7.2	5.8	6.4	10.2	8.6	9.2	5.1	7.7	5.5	16.2	20.1	16.8
Mortality (%)	30	25	-	43	38	-	21	40[a]	-	20	27	-

[a]Statistically significant with χ-test for equality of proportions (P=0.01) when compared with mortality in males.

TABLE 2

REPRODUCTIVE PERFORMANCE OF P GENERATION FEMALES

DELIVERED BY CAESAREAN SECTION

TREATMENT GROUP	NUMBER PREGNANT DAMS	MEAN DAYS TO PREGNANCY	MEAN NUMBER RESORPTIONS	MEAN NUMBER FETUSES/ LITTER	MEAN NUMBER DEAD FETUSES/ LITTER	RATIO OF MALES/ FEMALES	MEAN FETAL Wt (g)
H_2O Controls I & II	40	3.2	0.39	13.0	0	0.91	1.66
Coffee:							
12.5%	20	2.9	0.15	12.2	0	0.89	2.10
25.0%	20	4.0	0.10	12.2	0	0.76	1.91
50.0%	20	2.6	0.30	12.1	0	1.08	2.13
Caffeine:							
-Tube							
30 mg/kg	20	4.5[a]	0.27	12.2	0	0.88	2.01
-Water							
∿30 mg/kg	20	5.2[a]	0.20	12.9	0.15	0.88	1.67
Aspirin:							
-Tube							
125 mg/kg	20	5.5[a]	0.35	12.0	0	1.13	2.12

[a]Statistically significant with Student-t test (P=0.05).

TABLE 3

REPRODUCTIVE PERFORMANCE OF P GENERATION FEMALES

ALLOWED TO DELIVER NORMALLY

TREATMENT GROUP	NUMBER OF LITTERS	BIRTH			DAY 38	
		MEAN NUMBER LIVE F_1 PUPS/ LITTER	MEAN NUMBER DEAD F_1 PUPS/ LITTER	MEAN Wt OF F_1 PUPS (g)	NUMBER F_1 PUPS/ LITTER	MEAN Wt OF F_1 PUPS (g)
H_2O Controls I & II	10	11.6	0	6.5	11.1	119.0
Coffee:						
12.5%	5	10.2	0	6.4	9.4	123.6
25.0%	5	11.0	0	6.8	11.0	122.3
50.0%	5	11.0	0	6.5	10.6	108.3
Caffeine: -Tube						
30 mg/kg	5	14.2	0.4	5.9	11.6	115.1
-Water						
~30 mg/kg	5	11.6	0	7.3	7.4	119.4
Aspirin: -Tube						
125 mg/kg	5	11.2	0	7.0	10.6	119.6

TABLE 4

BODY WEIGHT AND SIZE AND ORGAN WEIGHTS OF REPRESENTATIVE F_1 FETUSES

DELIVERED BY CAESAREAN SECTION FROM TREATED DAMS

TREATMENT GROUP OF DAM	NUMBER OF FETUSES EXAMINED	MEAN BODY WEIGHT[a] g	MEAN FETAL SIZE		MEAN ORGAN WEIGHT[a] AS PERCENT OF BODY WEIGHT				
			LENGTH cm	WIDTH cm	BRAIN	LUNGS	HEART	LIVER	KIDNEYS
H_2O Controls									
I & II	167	1.662	2.543	.892	6.1	2.8	1.2	7.6	.65
Coffee:									
12.5%	79	2.099	2.701[b]	.942[b]	5.7	2.8	1.1	7.6	.65
25.0%	84	1.911	2.480	.908	5.9	2.7	1.2	7.5	.62[b]
50.0%	81	1.328	2.668[b]	.940[b]	5.4[b]	2.6[b]	1.1	7.0[b]	.64
Caffeine:									
-Tube									
30 mg/kg	81	2.008	2.603	.910	5.8	2.7	1.1	7.2[b]	.64
-Water									
~30 mg/kg	85	1.885	2.523	.844[b]	6.0	2.6[b]	1.1	7.4	.64
Aspirin:									
-Tube									
125 mg/kg	68	2.119	2.657[b]	.939	5.3[b]	2.6[b]	1.3[b]	7.8	.62

[a] Fetal and organ weights as determined after fixation in Bouin's solution.

[b] Statistically significant (P=0.05) with Student-t test.

TABLE 5

SUMMARY OF SOFT TISSUE ANOMALIES IN F_1 FETUSES

TREATMENT GROUP	TOTAL NUMBER FETUSES EXAMINED	NUMBER OF FETUSES SHOWING					
		HYDRO-CEPHALY	CLEFT PALATE	KIDNEY PELVIS UNDER-DEVELOPED	CRYPT-ORCHISM	CROOKED TAIL	EAR PINNA NOT FUSED
H_2O Control I & II	423	19	1	78	3	35	8
Coffee:							
12.5%	207	10	8[b]	45	1	16	8
25.0%	225	1	5[a]	58[a]	2	8	1
50.0%	229	8	4[a]	65[b]	4	6	2
Caffeine: -Tube							
30 mg/kg	252	0	1	61	8[a]	3	6
-Water							
~30 mg/kg	245	10	2	72[b]	7[a]	8	7
Aspirin: -Tube							
125 mg/kg	256	4	6[b]	101[b]	4	7	4

[a]Statistically significant (P=0.05) when analyzed with the ζ-test for equality of two proportions.

[b]Statistically significant (P=0.01) when analyzed with the ζ-test for equality of two proportions.

TABLE 6

SUMMARY OF SKELETAL ANOMALIES IN F_1 FETUSES

	H_2O CONTROLS I & II	COFFEE			CAFFEINE		ASPIRIN
		12.5%	25%	50%	INTUBATION (30 mg/kg)	WATER (~ 30 mg/kg)	INTUBATION (125 mg/kg
Number Fetuses Examined	256	128	141	148	171	160	188
Number Fetuses with Anomalies of:							
Any Ossification Abnormality	13	8	62[b]	11	12	15	15
Ribs -							
Focally Irregular Ossification	0	1	8[b]	0	9[b]	4[b]	13[b]
14th Unilateral	2	3	0	6[a]	0	3	0
Humerus -							
Incomplete Ossification	0	0	1	0	1	0	3[a]
Short	0	0	0	0	0	0	5[b]
Vertebra -							
Bipartite Ossification	2	2	2	4	9[b]	9[b]	11[b]
Fibula -							
Short	0	0	4[a]	0	0	0	0
Bowed	0	0	0	0	0	0	8[a]
Parietal -							
Incomplete Ossification	0	0	6[b]	8[b]	0	13[b]	1
Supraoccipital -							
Incomplete Ossification	0	8[b]	62[b]	11[b]	12[b]	15[b]	15[b]
Absent	0	0	0	0	10[b]	0	0

[a] Statistically significant (P=0.05) when analyzed with the χ-test for equality of two proportions.

TABLE 7

ESTIMATION OF THE DENSITY OF ALIZARIN RED-STAINED FETAL HUMERI

TREATMENT GROUP	NUMBER FETUSES EXAMINED	NEUTRAL FILTER		RED FILTER	
		AMOUNT OF LIGHT ABSORBED BY HUMERUS ($\bar{M}$ %)	TREATED/CONTROLS (%)	AMOUNT OF LIGHT ABSORBED BY HUMERUS ($\bar{M}$ %)	TREATED/CONTRO (%)
H_2O Controls I & II	256	19.2	-	24.6	-
Coffee:					
12.5%	128	17.3[a]	90.1	18.3[b]	74.4
25.0%	141	15.1[b]	78.6	16.6[b]	67.5
50.0%	148	14.9[b]	77.6	16.2[b]	65.8
Caffeine:					
-Tube					
30 mg/kg	171	16.2[b]	84.4	17.5[b]	71.1
-Water					
~30 mg/kg	160	15.6[b]	81.2	17.8[b]	72.4
Aspirin:					
-Tube					
125 mg/kg	188	16.4[b]	85.4	15.4[b]	62.6

[a]Statistically significant with Student-t test (P=0.05) when compared with Controls.

[b]Statistically significant with Student-t test (P=0.01) when compared with Controls.

TABLE 8

REPRODUCTIVE PERFORMANCE OF "UNTREATED" F_1 GENERATION

FEMALE RATS FROM TREATED P GENERATION FEMALES

P GENERATION TREATMENT GROUP	F_1 FEMALES		F_2 PUPS				
	% ACHIEVING PREGNANCY	MEAN DAYS TO PREGNANCY	MEAN NUMBER/ LITTER	MEAN Wt AT BIRTH g	MEAN % DEAD AT BIRTH	MEAN % SURVIVING TO DAY 28	MEAN Wt AT DAY 28 g
H_2O Controls I & II	70.5	5.3	12.6	6.9	2.2	91.0	64.1
Coffee:							
12.5%	64.5	6.4	12.0	6.4	0	95.4	61.8
25.0%	66.7	7.2[a]	13.2	6.4	0	94.4	64.3
50.0%	62.5	8.2[a]	12.8	6.6	0.4	95.0	66.4
Caffeine: -Tube							
30 mg/kg	57.6	9.6[a]	13.0	6.6	0	84.5	72.6
-Water							
∿30 mg/kg	57.1	14.3[a]	13.7	6.6	0	95.8	62.2
Aspirin: -Tube							
125 mg/kg	52.5	9.1[a]	12.2	6.6	0.4	92.0	61.3

[a] Statistically significant with Student-t test (P=0.05).

TABLE 9

MEAN CALCIUM CONCENTRATIONS IN BONES OF RATS EXPOSED TO 50% COFFEE IN UTERI AND WHICH LATER CONSUMED REGULAR COFFEE AS THEIR ONLY BEVERAGE FOR ONE YEAR

(% in Ash)

Sex[a]	Bones Analyzed	H_2O Control I		H_2O Control II		H_2O Controls I & II		Coffee 25%		Coffee 50%		Coffee 100%
		$\bar{M}$	SD	$\bar{M}$	SD	$\bar{M}$	SD	$\bar{M}$	SD	$\bar{M}$	SD	$\bar{M}$
Males	Foreleg	37.0	2.43	38.0	1.44	37.5	2.01	37.0	1.02	37.2	1.00	36.6
	Hindleg	36.3	2.24	38.0	1.00	37.1	1.94	38.1	4.62	37.4	0.80	37.0
	Skull	38.1	3.96	39.0	4.94	38.5	4.38	36.3	1.49	38.6	4.50	37.0
	F+H+S	111.7	4.98	115.0	6.22	113.5	5.76	111.4	6.10	113.2	5.55	110.6
Females	Foreleg	37.7	0.87	39.1	3.14	38.4	2.36	37.2	0.77	37.5	1.93	36.1[d]
	Hindleg	37.7	1.39	38.0	1.68	37.8	1.49	38.0	1.49	38.0	1.65	36.0[d]
	Skull	37.8	2.90	37.3	3.68	37.5	3.23	37.2	1.76	38.7	3.05	36.6
	F+H+S	113.2	2.85	114.4	5.54	113.7	4.30	112.4	3.29	114.2	3.44	108.8[bcd]

[a]Number rats examined = 10/sex/level.

[b]Statistically significant with Student-t test (P=0.05) when compared with Control I.

[c]Statistically significant with Student-t test (P=0.05) when compared with Control II.

[d]Statistically significant with Student-t test (P=0.05) when compared with Control I & II.

FOOTNOTES

1. Presented in part at the 15th Annual Meeting, Society of Toxicology, March 14-18, 1976, Atlanta, Georgia.

2. This work, conducted at Arthur D. Little, Inc., was funded by members of the National Coffee Association.

3. Present address: 83 Cockburn Street, Richmond, Ontario.

4. West Bend, Model #3600.

5. Paper in preparation by the Scientific Advisory Group, National Coffee Association.

6. WARF Institute, Inc., Madison, Wisconsin.

7. ATCO Manufacturing Company, Inc., Napa, California.

8. Lot #V1270 produced by Schwarz/Mann, Division of Becton, Dickinson Company.

9. Lot #70131 produced by the Merck Chemical Division.

10. The Charles River Breeding Laboratories, Wilmington, Massachusetts

11. Gloucester Rabbitry, Chepathet, Rhode Island.

12. Dennen Animal Industries, Inc., Gloucester, Massachusetts.

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Please add to GRAS Scientific Literature Review on Caffeine.

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